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Name _____ Date February 26/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Cech et al.

Filing Date: November 2, 1999

Serial No: 09/432,503

Docket: 018/063c

Title: INCREASING THE PROLIFERATIVE CAPACITY OF CELLS USING TELOMERASE REVERSE TRANSCRIPTASE

Art Unit: 1652

Examiner: Delia M. Ramirez, Ph.D.

COPY

DECLARATION UNDER 37 CFR § 1.132

JOHN M. IRVING, Ph.D.

Commissioner for Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, JOHN IRVING, do hereby declare as follows:

1. I am the Director of Molecular Biology at Geron Corporation. I have been working in the field of recombinant nucleic acid chemistry and vector construction for about 25 years. A copy of my *curriculum vitae* is enclosed with this Declaration. At Geron, I have been responsible for overseeing several different projects, such as PCR assay of gene expression in mammalian cells, and construction of viral vectors for transduction of mammalian cells, both i*n vitro* and *in vivo*.

2. I have read parts of the application referred to above that relate to construction of vectors for expressing human telomerase reverse transcriptase (hTRT). I have also read parts of patent application 10/143,536, relating to the adenovirus used in the rabbit ischemic ear model for wound healing. My group was responsible for constructing the adenovirus vector used in that study.

3. I understand the Examiner has questioned whether someone reading the application referred to above would know how to construct a vector similar to that used in the 10/143,536 application at the time that the disclosure of the present application was first filed in November 19, 1997.

4. The present application discusses various aspects of vector construction and its use in gene therapy: for example, on pages 65-82 and 110-114. Nonviral expression systems such as plasmids and episomal vectors are explained, and the reader is directed to a commercial supplier as a source of components. Viral vectors are also explained, including those based on retrovirus, adenovirus, AAV, and other systems in common use.

These systems were well known and described at the time the application was filed. See the following publications as an illustration of contemporary techniques for adenovirus and retrovirus construction:

- Graham & Prevec. Adenovirus-based expression vectors and recombinant vaccines. Biotechnology 290:363, 1992.
- Sime, Xing, Foley, Graham & Gauldie. Transient gene transfer and expression in the lung. Chest 111:89S, 1997.
- He, Zhou, da Costa, Yu, Kinzler & Vogelstein. A simplified system for generating recombinant adenoviruses. Proc. Natl. Acad. Sci. USA 95:25009, 1998.
- Anton & Graham. Site-specific recombination mediated by an adenovirus vector expressing the Cre recombinase protein: a molecular switch for control of gene expression. J. Virol. 69:4600, 1995.
- Hitt, Addison & Graham. Human adenovirus vectors for gene transfer into mammalian cells. Adv. Pharmacol. 40:137, 1997.
- Graham et al. Adenovirus vectors for gene therapy. PCT publications WO 95/00655 and WO 96/40955 (now U.S. patents 5,919,676; 6,120,764; and 6.140,087, assigned to Advec, Inc).

- Pear, Nolan, Scott & Baltimore. Production of high-titer helper-free retroviruses by transient transfection. Proc. Natl. Acad. Sci. USA 90:8392, 1993.
- Kinsella & Nolan. Episomal vectors rapidly and stably produce high-titer recombinant retrovirus. Hum Gene Ther 7:1405, 1996.
- Lindemann, Patriquin, Feng & Mulligan. Versatile retrovirus vector systems for regulated gene expression *in vitro* and *in vivo*. Mol Med 3:466, 1997.
- Sadelain, Wang, Antoniou, Grosveld & Mulligan. Generation of a high-titer retroviral vector capable of expressing high levels of the human β-globin gene. Proc. Natl. Acad. Sci. USA 92:6728, 1995.

5. If I wanted to make an hTRT expression vector in 1997, based on the discussion in the disclosure, I would have proceeded according to the same principles of vector construction that would be employed by any reader with appropriate experience.

A typical procedure might be as follows: two plasmids would be prepared by recombinant DNA techniques using bacterial cells. One would contain the adenovirus backbone, missing the left-hand ITR and the E1 region, but including the rest of the adenovirus encoding region up to the right-hand ITR. The other would be a small shuttle plasmid. It would contain the left-hand ITR and packaging signals, followed by an expression cassette containing the hTRT encoding region under control of a suitable promoter replacing the E1 region. The expression cassette is flanked by two regions of 2 kb or more of adenovirus sequence shared with the backbone plasmid, permitting homologous recombination. The hTRT encoding sequence is provided in the disclosure, and could be cloned from any suitable library as described. A number of promoters would work. Many of the promoters listed on page 67 of the specification would be good choices.

The two plasmids would then be opened with a restriction nuclease, and co-transfected into a eukaryotic host cell that could supply E1 function in trans. 293 cells are typically used for this purpose. Suitable transfection protocols known at the time were the calcium-phosphate method, and the lipofection method. The plasmids would assemble together in the host cell by homologous recombination, replicate in the cytoplasm, and be packaged into replication-incompetent adenovirus vectors.

6. In the 10/143,536 application, an hTRT adenovirus vector is described in Example 4, and then used in the ischemic ear model in Example 6. This adenovirus vector was constructed by the vector group at Geron Corporation under my supervision. We used the commercially available AdMax™ system obtained from Microbix Biosystems Inc., Toronto Canada, which distributes kits on behalf of Advec, Inc. This system again uses an adenovirus backbone plasmid and a shuttle plasmid. Recombination is effected not by homologous regions in the plasmid, but by site-specific recombination using the Flp/*frt* system which is somewhat more efficient.

Use of site-specific recombinase enzymes for assembling adenoviruses were already known and in use (WO 96/40955) at the time the present disclosure was first filed. Transcription is driven in our hTRT vector by the CAG system, containing the CMV enhancer and a modified chicken beta-actin promoter. The CAG system was also known (Kiwake, Endo et al., Hum Gene Ther 7:821, 1996) at the time this disclosure was first filed.

7. The adenovirus vector made using the AdMax™ system for the 10/143,536 application contains an hTRT expression cassette in essentially the same functional arrangement as the vector made by homologous recombination in the manner described above. A vector made by homologous recombination would be expected to perform in the experiments described in the 10/143,536 application in a similar fashion.

8. I hereby declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

1-31-03
Date

John M. Irving
John M. Irving, Ph.D.
Menlo Park, CA